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The Effects of Thiourea Treatment Upon the Thyroid, Pituitary and Gonads of the Zebra Fish, *Brachydanio rerio*[1]

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(Plate I)

INTRODUCTION

SINCE the work of Richter & Clisby (1941) and MacKenzie & MacKenzie (1942), thiourea and related antithyroid chemicals have become widely used as a tool for the study of the thyroid and its physiological relationship with other endocrine glands of the body. Investigations upon the common laboratory mammals have revealed essentially consistent results. The treated animals responded to this method of inhibiting the synthesis or release of the thyroid hormone in several ways. Growth and differentiation were retarded particularly in young animals, cellular changes were produced in several of the endocrine organs, the most noticeable of these being the increased number of basophilic cells in the anterior lobe of the pituitary. The increase in the gross weight of the thyroid gland was associated with cellular hypertrophy, follicular hyperplasia and colloid loss.

Some of these effects have been produced in the lower vertebrates, including the teleost fishes. Goldsmith *et al.* (1944) found that immersion of a hybrid strain of *Xiphophorus helleri* and *Xiphophorus* (*Platypoecilus*) *maculatus* in thiourea solutions resulted in the inhibition of growth, failure in the development of the secondary sex characters and marked hyperplasia of the thyroid gland. A retardation in the maturation of the gonads, as well as in the development of secondary sex characters, was correlated with thyroid changes in *Phoxinus laevis* following chemical thyroidectomy (Barrington & Matty, 1952). Similar thiourea treatment of the characin, *Astyanax mexicanus*, by Rasquin (1949) did not produce significant changes in the percentage of chromophilic cells in the pituitary gland.

The experiments presented here were designed to determine the effects of thiourea upon the thyroid, pituitary and gonad of the freshwater teleost, *Brachydanio rerio* (Hamilton-Buchanan) (the zebra fish of tropical fish fanciers), as well as its effect upon body growth..

Acknowledgement is made at this time to Dr. J. Walter Wilson, under whose guidance this investigation was undertaken.

MATERIALS AND METHODS

Young zebra fish of known ages were raised from eggs laid by a single female fish and fertilized by one or two males. A total of 50 young fish were selected for a similarity in their total lengths and set up experimentally, as shown in Table 1.

Each group of fish was kept in a bare five-gallon tank which was equipped only with a conventional electric heating unit to maintain water temperature at 76°-78° F., and a porous stone that was connected to an air line for aerating and circulating the water. The two groups of control fish were kept in conditioned tap water while the treated fish were immersed in 0.33% thiourea solutions prepared with conditioned tap water. During the first month of treatment, the solutions of the control and treated tanks were changed bi-weekly. After this period, changes were made weekly.

At the end of each experiment the fish were stunned either by immersion in iced water or

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TABLE 1. SUMMARY OF TREATMENT OF ZEBRA FISH BY IMMERSION IN 0.33% SOLUTIONS OF THIOUREA*

	Number of Animals	Mean Length (mm)	Age (Weeks)	Length of Treatment (Weeks)	Mortality
			Experiment 1		
Control	15	10.6	6	16	1
Treated	15	10.7	6	16	4
			Experiment 2		
Control	10	23.0	20	14	1
Treated	10	23.3	20	14	3

* For details see Table 4.

chilled Bouin's solution, quickly measured for total lengths on graph paper and divided into three portions by two transverse cuts—one made at the posterior margin of the operculum and the second at the posterior extremity of the body cavity. The head and body pieces were then fixed in Bouin's solution. Tissues were dehydrated in Dioxane and infiltrated and embedded in 56°-58° paraffin. Transverse serial sections, five micra in thickness, were obtained from the head piece and sections of seven micra thickness from the body piece.

Sections of the head region which included the pituitary gland were stained with Masson's connective tissue stain (Lillie, 1940), using acid fuchsin-ponceau xylidin mixture and fast green F. All other sections were stained with Harris's hematoxylin and counterstained with eosin.

Measurements of the height of the thyroid epithelial cells were made according to the method of Rawson & Starr (1938) with certain necessary modifications. Their method, developed with mammalian thyroid studies, consisted of measuring the height of one cell for each of 200 different follicles. Instead of this, because of the small size of the zebra fish thyroid, ten sections containing thyroid tissue were selected for analysis at intervals of 90 micra or more. Ten cells were selected for measurement from all of the follicles present in each section. For the controls, an average of three cells were measured from each follicle; while for the treated, only one cell for each follicle was required. In this way, a total of 100 measurements was made for each fish. The anterior-posterior length of the area of distribution of the thyroid follicles was determined by analysis of the serial sections of the lower jaw.

Cell counts were made of the transitional lobe of the pituitary, employing the technique used by Rasquin (1949). A Howard ocular marked in 16 equal squares was used as a counting chamber and all cells that fell within the 16 squares were counted. By moving the slide carefully, it was possible to count all other fields in the same section of the transitional lobe without duplication. Beginning with the section in which the transitional lobe first appeared, all the cells with nuclei were counted for every 2nd, 3rd or 4th section, depending upon the size of the fish.

The gonads of the treated fish were also carefully compared histologically with those of the controls, in order to determine whether the treatment had any effect upon these organs.

The total lengths of treated and control fish were analyzed statistically according to the Fisher modification of "Student's" formula for small samples:

$$t = (\overline{V}_1 - \overline{V}_2)\sqrt{\frac{n_2 + n_2 - 2}{n_1\sigma_1^2 + n_2\sigma_2^2}}\sqrt{\frac{n_1 n_2}{n_1 + n_2}}$$

This formula was also employed to test the significance of the results obtained from the study of the thyroid follicular epithelium and from the numerical comparison of pituitary basophilic cells.

RESULTS

Effects of Treatment upon Thyroid.—The zebra fish thyroid, like that of other teleosts (Gudernatsch, 1911), consists of a number of individual follicles scattered in a long, narrow streak, ranging from the first to the third branchial artery and loosely suspended in the connective tissue of the lower jaw around the ventral aorta. The anterior limit of the zebra fish thyroid is usually prominently marked by the largest follicle of the whole gland, and it is abreast of the forward extremity of the gill chambers. Follicles are lined by a single layer of epithelium whose cells vary from a very flat squamous-like cell to a cuboid one (Pl. I, Fig. 1).

The changes caused in this gland by thiourea treatment were of a hyperplastic nature. The

most striking effect was the increase in number of small follicles throughout the length of the gland. The smaller blood vessels supplying the thyroid became distended with blood and contributed to the crowded condition of the lower jaw. The anterior-posterior axis of the gland was increased almost two-fold over that of the controls (Table 2). New areas were invaded by the numerous small follicles in order to accommodate the enlarged thyroid. Follicles were observed wedged between the bones of the anterior end of the jaw, arranged around the branchial arteries in the gill arches and embedded in tissues on the anterior surfaces of the heart.

A marked increase in cellular height of the follicular cells was produced (Pl. I, Fig. 2). Table 2 shows that this effect was more pronounced in the older group, where the increase was from 2.6 micra to 4.8 micra. The nuclei of these cells also increased in size, became more vesicular and usually contained recognizable nucleoli. In the older group of fish, colloid loss was indicated by a few individual collapsed follicles scattered throughout the gland, but this was most often made apparent by the vacuolated condition of the colloid of the larger follicles (Pl. I, Fig. 2). In the other group of treated fish, smooth acidophilic colloid predominated and collapsed follicles were not observed.

Effects of Treatment Upon the Pituitary Gland.—As has been reported for other teleosts (Potts, 1942, and Levenstein, 1939), there are four portions of the zebra fish pituitary gland and they are distinguishable mainly by the staining reactions of their cells. These are referred to as the pars nervosa, pars anterior, pars intermedia and the transitional lobe or ubergansteil. No distinct septa separate these portions. Most of the central portion of the gland is composed of the large transitional lobe whose mid-dorsal border is pushed inward by ramifications of nerve fibers and blood vessels of the pars nervosa. Anterior and ventral to this lies the pars anterior. The pars intermedia is a shell-like portion located immediately behind the transitional lobe, thus constituting the posterior portion of the gland.

With Masson's trichrome stain, the transitional lobe contrasted sharply with the cellular elements of the other portions of the pituitary, because it contained intensely stained dark red acidophilic cells, bright green basophilic cells and colorless or light orange chromophobic cells. In the glands of all fish, the chromophobic cells were not numerous and the basophilic cells outnumbered the acidophilic ones. The peripheral regions of the transitional lobe were usually packed solidly with basophilic cells, while the central portion contained most of the acidophilic cells, arranged in clusters which often surrounded the small blood vessels of the gland.

The most striking effect of thiourea treatment upon the pituitary of the zebra fish was a marked increase in the percentage of basophilic cells in the transitional lobe, as is shown in Table 3. Mitotic figures were observed among the basophilic cells, but no increase in cellular size was detected. The vascularity of the gland, as a whole, appeared to be slightly increased.

Effect of Treatment Upon the Gonads.—The gonad development of the younger group of treated fish was much retarded by thiourea treatment, while in the older group, with one exception, maturation of the testes was not effected.

After sixteen weeks of treatment, the fish in the younger group were twenty-two weeks old. The gonads of the control fish were easily identified as testes or ovaries by microscopic examination. The ovaries were composed of developing eggs of varied ages and sizes (Pl. I, Fig. 4). The testes consisted of cysts of cells in all stages of spermatogenesis, but earlier stages predominated (Pl. I, Fig. 3). In contrast with this, all gonads of the treated fish were much smaller in size and showed little or no cellular differentiation. The major portion of all these gonads was composed of a few gonial cells, which were encircled and held together either singly or in occasional groups of two or more cells by interstitial cells (Pl. I, Fig. 5). The cellular appearance of these cells in the gonads of treated fish was similar to that of the early developmental stages observed by Frolander (1950). Each gonial cell contained a large round oval nucleus, 4-6 micra in diameter, with a dark basophilic-staining nucleolus, and evenly distributed, fine chromatin granules in its nucleoplasm. The cystoplasm of these cells was moderately stained and filled with very fine granules. In the gonads of only two specimens of this group some development had occurred, and here a few cysts of cells were observed as recognizable stages of spermatogenesis. Even in these organs the gonial cells were predominant, however.

All of the treated fish belonging to the older group proved to be males and therefore limited the observations of the effect of thiourea to the testis. Comparable numbers of cysts of all stages of spermatogenesis were present in both the treated and control fish. The majority of the testicular cysts in all testes, treated and control, were in the late states of spermatogenesis. There was a single example of testis involution where the treated gonad consisted of cysts of typical gonial cells (Pl. I, Fig. 6). An exhausted or spent condition of this testis was indicated also by the

presence of a few cysts of sperm cells and the absence of any intermediate stages of spermatogenis.

Effect of Treatment on Growth.—Another effect of the thiourea treatment was a retardation of growth. Table 4 shows this comparison of total lengths of treated and control fish. The Fisher modificaton of "Student's" formula was applied for testing the significant differences between groups, since small numbers of animals were used for experimentation. Values for t (significance) of 3 or higher indicate that results are significant. In this experiment, values of 12.01 and 3.48 were obtained.

Discussion

Thyroid.—Some of the changes produced in the thyroid of the zebra fish after thiourea treatment were a readily apparent increase in the number of follicles visible in a single field, a preponderance of very small follicles, an increased height of follicular cells and a widespread proliferation of follicles with an increase in thyroid length and an invasion into abnormal regions of the lower jaw. Some of these effects were found in the thyroids of the fish of a *Xiphophorus* hybrid strain (Goldsmith *et al,* 1944). The thyroid region of the lower jaw also became hyperemic, very much like the condition described by Tinacci (1948) for selachians after thiouracil ingestion and also similar to that produced in *Fundulus* by injections of sheep pituitary extracts (Albert, 1945).

However, the hyperplastic changes were not as severe as those that have been produced in higher forms. A regression of thyroid activity, as was evidenced by the presence of numerous small follicles with non-vacuolated colloid and the scarcity of collapsed follicles, was more typical of the results obtained after long (4 months) thiourea exposures of tadpoles (Gordon *et al,* 1945) and after longer (12 months) thiouracil treatment of mice (Dalton *et al,* 1948). This suggests either a failure in thyrotropic hormone

Table 2. Length of Thyroid Gland and Cellular Heights of the Follicular Epithelium

Experiment 1

Twenty-two-weeks old fish immersed in 0.33% solutions of thiourea for 16 weeks.

	Gland Length			Follicular Epithelium		
Fish	Total Length (mm)	Thyroid Length (micra)	Thyroid Length per mm of Total Length	Cellular Height (micra)*	Standard Error (S.E.)	Significance (t)
			Controls†			
1	24	1071	44	2.40		
2	24	1358	57	1.96		
3	24	—	—	2.16		
4	24	1099	46	2.29		
5	24	1190	49	2.23		
6	24	1694	71	2.33		
7	24	1302	54	2.23		
10	25	1386	55	2.28		
11	26	1757	65	2.68		
Average	24.4	1208	55	2.28	±0.08	5.02
			Treated			
1	14	1649	118	3.94		
2	15	1855	124	4.27		
3	15	1354	90	4.58		
4	15	1312	89	3.01		
5	16	1922	120	2.21		
6	17	2027	119	4.79		
7	17	—	—	3.72		
8	17	2044	120	2.81		
9	17	1583	93	3.50		
10	15	1554	102	3.81		
11	15	1554	102	3.28		
Average	15.7	1685	108	3.63	±0.22	

production or a failure in the hormone release mechanism of the pituitary gland, or a combination of both. The fact that the evidence for thyroid regression was more prominent in the younger, longer-treated fish seems to indicate a more complete failure of the pituitary glands of this group of fish.

Pituitary.—Evidence of a thyroid-pituitary relationship in teleosts has beeen presented by experiments of a varied nature. In 1940, Gorbman increased the height of the follicular epithelium of the goldfish thyroid gland by injections of thyrotropic hormones from a variety of sources. More recently, Olivereau noted that there was a distinct difference in thyroid activity between two species of *Mullus*. The histological appearance of the pituitary of these two species reflected the state of activity of the thyroid glands. Increased numbers of pituitary basophilic cells were correlated with a hyperfunctional thyroid and thus provided a basis for the conclusion that these cells were concerned in the formation of the thyrotropic hormone in these teleosts.

The results of the histological study of the pituitary and thyroid of the zebra fish after thiourea treatment support this conclusion. In this animal an increase in the percentage of basophilic cells of the pituitary is correlated with the hyperactivity of the thyroid and indicates that in the zebra fish these cells are the components of the pituitary gland concerned with the production and secretion of the thyrotropic hormone. As in other forms, the thyroid gland is rendered hypofunctional by thiourea. To correct for this condition, the basophilic cells of the pituitary produce and secrete abnormal amounts of tropic hormones which, in turn, cause the thyroid to become hyperactive. This gland, however, because of continued treatment, remains hypofunctional.

Gonads.—The effects of antithyroid substances upon gonads of higher forms seem to fall into two catagories. If thiourea is admin-

TABLE 2. LENGTH OF THYROID GLAND AND CELLULAR HEIGHTS OF THE FOLLICULAR EPITHELIUM (*Continued*)

Experiment 2

Thirty-four-weeks old fish immersed in 0.33% solutions of thiourea for 14 weeks.

	Gland Length			Follicular Epithelium		
Fish	Total Length (mm)	Thyroid Length (micra)	Thyroid Length per mm of Total Length	Cellular Height (micra)*	Standard Error (S.E.)	Significance (t)
			Controls†			
1	28	2226	79	2.64		
2	30	2415	80	3.19		
3	27	2037	75	2.64		
4	25	1650	66	2.97		
5	28	1809	64	2.86		
6	29	2177	74	2.31		
7	28	1918	67	2.31		
8	29	1960	67	2.20		
9	23	1771	77	1.87		
Average	27.4	1996	72	2.55		
					±0.13	9.19
			Treated			
1	25	2982	119	5.72		
2	25	2485	99	4.29		
3	25	3101	124	4.29		
4	22	2394	108	4.29		
5	25	3213	128	4.84		
6	25	2772	111	4.73		
7	24	3507	146	5.17		
Average	24.4	2922	120	4.76		
					±0.19	

* This figure represents the average of measurements of 100 cells from follicles of 10 different sections of the gland. The height was taken for 10 follicle cells from each section, the sections being approximately 160 micra apart.

† Since such consistent results were obtained from the study of thyroid glands of the control fish, only nine of the fourteen were utilized.

istered for short periods, no changes are produced in the adult frog testis (Joel *et al,* 1949). However, inhibition of gonad development, as well as involution of gonads, have been reported after prolonged (20 weeks) thiourea treatment in mice (Dalton *et al,* 1949) and in chickens (D'Angelo *et al,* 1948). In young *Xiphophorus* hybrids (Goldsmith *et al,* 1944) and young *Lebistes* (Nigrelli *et al,* 1946) prolonged (4 months) thiourea treatment completely prevented the development of the secondary sex characters. In the minnow, *Phoxinus laevis,* the development of the gonads as well as the appearance of secondary sex characters was retarded by this means of chemical thyroidectomy (Barrington & Matty, 1952). On the other hand, conflicting results are recorded for thyroid excess experiments. A precocious development of the gonopodium (modified anal fin) was induced in immature male platyfish, *Xiphophorus* (*Platypoecilus*) *maculatus,* with either thyroid powder or thyroxine solution (Grobstein & Bellamy, 1939). However, Smith & Everett (1943) were unable to induce similar changes in guppies fed similar substances from birth.

In the zebra fish, inhibition of gonad development and differentiation was complete in the younger group where treatment was initiated upon fish with undifferentiated gonads, and this effect is parallel to, if not more striking than, those produced in *Phoxinus laevis,* another member of the family Cyprinidae (Barrington & Matty, 1952). On the other hand, in the testes of the older group of zebra fish where treatment was begun on immature but differentiated gonads, thiourea seemed to have little or no effect. Although a statistical study was not made, there appeared to be comparable numbers of cysts of all stages of spermatogenesis in both the control and treated fish. The aberrant condition of the testes of the single responsive male of this same group is very similar to that

Table 3. Percentage of Basophilic Cells of the Pituitary

Experiment 1

Twenty-two-weeks old fish immersed in 0.33% solutions of thiourea for 16 weeks.

(All of the acidophilic and basophilic cells were counted in every third section of the gland in which the transitional lobe was present for the controls, and in every second section for the treated).

Fish	Total Length (mm)	Sex	Total Cells Counted	Number of Basophilic Cells	Per cent Basophilic Cells	Average	Standard Error (S.E.)	Significance (t)
			Controls					
1	24	M	Poor histological preparation					
2	24	F	5470	3092	56.5			
3	24	F	3668	2162	59.0			
4	24	M	5437	3794	69.0			
5	24	M	3665	2229	60.9			
6	24	F	4085	2450	60.1			
7	24	F	2347	1308	55.7	59.1%	±1.4	
8	25	F	3333	1816	54.5			
9	25	F	2822	1456	52.0			
10	25	M	2335	1459	62.6			
11	26	F	4076	2498	61.3			
12	27		Pituitaries not studied					
13	28		Pituitaries not studied					
14	28		Pituitaries not studied					9.42
			Treated					
1	14	U	Poor histological preparation					
2	15	U	1560	1114	71.4			
3	15	U	1531	1250	81.6			
4	15	U	1033	892	85.4			
5	16	U	1296	1111	85.7			
6	17	M	3238	2555	79.9	80.7%	±1.4	
7	17	U	1885	1487	78.8			
8	17	U	2720	2211	82.2			
9	17	M	2070	1691	81.7			
10	15	U	Entire gland stained lightly acidophilic					
11	15	U	Entire gland stained lightly acidophilic					

of a single male of the treated minnows. These results of gonad inhibition are similar to effects of hypophysectomy upon *Fundulus* gonads reported by Mathews, 1939. If the inhibition and involution effects of thiourea on the gonads are considered in terms of the changes produced in the thyroid gland, it can be postulated that all of these changes are induced by way of the pituitary gland and probably are not due to a direct action of the chemical. It is as though the increased demands for thyrotropin stimulated by thiourea treatment have overtaxed the pituitary to such an extent that amounts of gonadotropin, adequate for normal gonad development, are no longer maintained. Confirmation of the reduction of gonadotropin production during thiourea treatment is lacking in fish; but in rats, after short (20-22 days) treatments, evidence for larger-than-normal amounts of gonadotropin was reported by Leathem (1945), but after prolonged (8 months) treatment, lower-than-normal levels of this hormone were detected by Janes (1946).

Growth.—Thiourea treatment caused a retardation in growth of the zebra fish, just as it did in *Lebistes* and in *Xiphophorus*. This effect upon growth is more pronounced in the young, rapidly-growing zebra fish than in the more mature one. Since growth retardation is correlated with a hypofunctional thyroid, then normal growth rates in fish must be dependent, in part at least, upon the thyroid or its hormone. This relationship of the thyroid hormone to growth has been tested further in fish by Nigrelli *et al* (1946). Simultaneous treatment of guppies with mammalian thyroid powder and thiourea caused an increase in growth of these fish similar to that of their untreated controls and greater than that of thiourea-treated fish. In these experiments upon the zebra fish—while most of the effects upon growth must also have been by way of the thyroid gland—evidence of some liver damage suggests that some of the growth retardation may have been the result of the toxicity of the concentration of thiourea used.

Summary

1. The thyroid, pituitary and gonad of the tropical freshwater fish, *Brachydanio rerio,* were examined histologically to ascertain the effects of immersion in solutions of 0.33% thiourea. An analysis of total lengths of fish was also made as a means of observing effects upon growth. The evidence obtained from the observation of the glands indicated that such treatment produced a hypofunctional thyroid in the zebra fish.

Table 3. Percentage of Basophilic Cells of the Pituitary (*Continued*)

Experiment 2

Thirty-four-weeks old fish immersed in 0.33% solutions of thiourea for 14 weeks.

(All of the acidophilic and basophilic cells were counted in every fourth section of the gland in which the transitional lobe was present).

Fish	Total Length (mm)	Sex	Total Cells Counted	Number of Basophilic Cells	Per cent Basophilic Cells	Average	Standard Error (S.E.)	Significance (t)
				Controls				
1	28	F	6018	2968	49.3			
2	30	M	7477	4849	64.9			
3	27	M	4777	3363	70.4			
4	25	M	3194	2059	64.7			
5	28	M	7397	5397	72.0	67.5	±1.23	
6	29	M	5813	4085	70.2	(average		
7	28	M	4004	2711	67.7	% for 7		
8	29	M	6091	3811	62.6	males)		
9	23	F	3021	2332	77.0*			4.62
				Treated				
1	25	M	5833	4823	82.7			
2	25	M	5755	4664	81.2			
3	25	M	6606	5547	84.0			
4	22	M	3024	2624	72.7	77.9	±1.69	
5	25	M	3129	2229	74.0			
6	25	M	5865	4567	77.9			
7	24	M	4375	3198	73.1			

* Control specimen No. 9 was sickly and stunted when killed.

2. Thiourea caused thyroid hyperplasia, as was evidenced by a large increase in the numbers of small follicles; an increased length of the gland; the invasion of follicles into abnormal sites in the anterior region of the jaw, in the branchial region, and on the anterior surface of the pericardium; the hyperemic condition of the lower jaw; and the increased cellular heights of the follicular cells. The loss of colloid was not pronounced and usually was not associated with follicular collapse.

3. Cell counts showed that the changes in the pituitary consisted of an increase in the percentage of basophilic cells of the transitional lobe with some increase in vascularity of the entire gland. These results are consistent with the hypothesis that the basophilic cells are the source of the thyrotropic hormone in the zebra fish.

4. The thyroid gland of the zebra fish is concerned in maintaining a normal growth rate. By treatment, growth was retarded in both the six-weeks and five-months old groups of fish. More pronounced effects were shown by the young fish.

5. An inhibition of gonad development characteristic of the young treated fish and an instance of gonad involution in the older treated fish suggest that insufficient gonadotropic substances were being produced by a pituitary gland, seemingly overtaxed by the demands of a hypofunctional thyroid.

Literature Cited

Albert, A.

1945. The experimental production of exophthalmos in *Fundulus* by means of anterior pituitary extracts. Endocrin, 37: 389-406.

Table 4. A Comparison of Growth of Control and Treated Fish

Experiment 1

Twenty-two-weeks old fish immersed in 0.33% solutions of thiourea for 16 weeks.

Length at Beginning of Exp. (mm)	Average Length (mm)	Standard Error	Length at End of Exp. (mm)	Average Length (mm)	Standard Error	Significance (t)
			Controls			
10			24			
10			24			
10			24			
10			24			
10			24			
10			24			
11	10.60	±0.12	24	25.07	±0.59	
11			25			
11			25			
11			25			
11			26			
11			27			
11			27			
11			28			
11						12.01
			Treated			
10			14			
10			15			
10			15			
10			15			
11			15			
11			15			
11			16			
11	10.73	±0.24	17	15.72	±0.34	
11			17			
11			17			
11			17			
11						
11						
11						
11						

Barrington, E. J. W. & A. J. Matty
1952. Influence of thiourea on reproduction in the minnow. Nature, 170 (4316): 105-106.

D'Angelo, S. A., A. S. Gordon & H. A. Charipper
1948. Effects of thiourea on the growth, plumage, and endocrine organs in the fowl. Anat. Rec., 99: 663.

Dalton, A. J., H. P. Morris & C. S. Dubnik
1948. Morphologic changes in the organs of female C3H mice after long-term ingestion of thiourea and thiouracil. J. Nat. Cancer Inst., 9: 201-223.

Frolander, H. T.
1950. Normal gonadogenesis in the cyprinid fish, *Brachydanio rerio*. Master's Thesis, Brown University.

Goldsmith, E. D., R. F. Nigrelli, A. S. Gordon, H. A. Charipper & M. Gordon
1944. Effect of thiourea upon fish development. Endocrin, 35: 132-134.

Gorbman, A.
1940. Suitability of the common goldfish for assay of thyrotropic hormone. Proc. Soc. Exper. Biol. Med., 45: 772-773.

Gordon, A. S., E. D. Goldsmith & H. A. Charipper
1945. The effects of thiourea on amphibian development with a note on the ineffectiveness of sulfadiazine and para-amino benzoic acid. Growth, 9: 19-41.

Grobstein, C. & A. W. Bellamy
1939. Some effects of feeding thyroid to immature fish. Proc. Soc. Exper. Biol. Med., 41: 363-365.

Gudernatsch, J. F.
1911. The thyroid gland of the teleosts. J. Morph., 21: 709-782.

Janes, R. G.
1946. Further observations on the experimental production of ovarian cysts. Anat. Rec., 94: (Suppl.) 473. Abstract.

Joel, T., S. A. D'Angelo & H. A. Charipper
1949. The effects of thiourea on the thyroid gland of the winter frog (*Rana pipiens*) with some observations on the testis. J. Exper. Zool., 110: 19-29.

Leathem, J. H.
1945. Influence of thiourea on plasma proteins and organ weights in the rat. Endocrin., 36: 98-103.

Table 4. A Comparison of Growth of Control and Treated Fish (*Continued*)

Experiment 2

Thirty-four-weeks old fish immersed in 0.33% solutions of thiourea for 14 weeks.

Length at Beginning of Exp. (mm)	Average Length (mm)	Standard Error	Length at End of Exp. (mm)	Average Length (mm)	Standard Error	Significance (t)
			Controls			
20			23			
20			25			
22			27			
22			28			
23	23.00	±0.58	28	27.44	±0.71	
24			28			
24			29			
25			29			
25			30			
25						3.48
			Treated			
20			22			
22			24			
22			25			
23	23.30	±0.56	25	24.45	±0.27	
23			25			
23			25			
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Levenstein, Irving
1939. The cytology of the pituitary gland of two varieties of goldfish (*Carassius auratus* L.), with some reference to variable factors in the gland which may possibly be related to the different morphological types. Zoologica, 24: 47-60.

Lillie, R. D.
1940. Histopathologic Technique. The Blakiston Company, Philadelphia and Toronto.

MacKenzie, J. B. & C. G. MacKenzie
1942. Effect of "Sulfa" drugs on the thyroid glands in rats and mice. Federation Proc., 1: 122-123.

Mathews, S. A.
1939. The relationship between the pituitary gland and the gonads in *Fundulus*. Biol. Bull., 76: 241-250.

Nigrelli, R. F., E. D. Goldsmith & H. A. Charipper
1946. Effects of mammalian thyroid powder on growth and maturation of thiourea-treated fishes. Anat. Rec., 94: 79. Abstract.

Olivereau, M.
1948. Histologie comparée des hypophyses de *Mullus barbatus* L. et de *Mullus surmuletus* L. (Arch. Zool. Exp. et Gen., 85: 100-108) Biological Abstract, 23: No. 1511.

Potts, H. E.
1942. The anatomy and morphology of the hypophysis of several species of ovoviviparous poeciliids. Zoologica, 27: 85-96.

Rasquin, P.
1949. The influence of light and darkness on thyroid and pituitary activity of the characin *Astyanax mexicanus* and its cave derivatives. Bull. Amer. Mus. Nat. Hist., 93: 501-531.

Rawson, R. W. & P. Starr
1938. Direct measurement of heights of thyroid epithelium. Arch. Intern. Med., 38: 726-738.

Richter, C. P. & K. H. Clisby
1941. Graying of hair produced by ingestion of phenylthiocarbamide. Proc. Soc. Exper. Biol. Med., 48: 684-687.

Smith, D. C. & G. M. Everett
1943. The effect of thyroid hormone on growth rate, time of sexual differentiation and oxygen consumption in the fish, *Lebistes reticulatus*. J. Exper. Zool., 94: 229-240.

Tinacci, F.
1948. L'azione di alcune sostanze antitiroidee (Tiuracile, Metiltiuracile e Aminotiuracile). Publ. Staz. Zool. Napoli., 21: 124-131.

EXPLANATION OF THE PLATE

All figures are photomicrographs of tissues fixed in Bouin's and stained with haemotoxylin-eosin. × ca. 850.

Fig. 1. The flat epithelium and smooth acidophilic colloid of a thyroid follicle from a control fish 34 weeks old.

Fig. 2. The heightened epithelium and vacuolated colloid of a thyroid follicle from a 34-weeks-old fish after 14 weeks of thiourea treatment.

Fig. 3. A portion of the testis of a control fish 22 weeks old, showing many stages of spermatogenesis.

Fig. 4. A section from the right ovary of a control fish 22 weeks old.

Fig. 5. A section of an undifferentiated gonad of a 22-weeks-old fish, after 16 weeks of thiourea treatment. Note the few large nuclei surrounded by smaller, darker-stained, interstitial cells.

Fig. 6. A portion of an involuted testis from a 34-weeks-old fish, after thiourea treatment for 14 weeks. Note the numerous cells (similar to gonial cells) with large nucleolated nuclei.